EMBRYOLOGY, KARYOLOGY, AND MODES OF REPRODUCTION IN THE NORTH AMERICAN GENUS *BOECHERA* (BRASSICACEAE): A COMPILATION OF SEVEN DECADES OF RESEARCH[1]

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ABSTRACT

The North American–Greenlandic genus *Boechera* Á. Löve & D. Löve (Brassicaceae) is distinguished from other phylogenetic lineages recognized within the artificial taxon *Arabis* L. s.l., in which it has formerly been included, by its base chromosome number *x* = 7. Based on outgroup comparisons, we consider this chromosome number to be derived from an ancestral genome comprising eight chromosomes. Chromosome counts are now available for about half of the species names listed in the most recent taxonomic treatments of the genus. An analysis of the karyological differentiation with respect to chromosome numbers was performed, and these patterns were correlated with data on pollen viability and population genetic measures in order to deduce modes of reproduction. This approach allowed us to distinguish three main groups: sexual, amphi-apomictic, and apomictic species, respectively. We focused further on the cytology of gametes, especially on that of pollen, and we discuss its role in the formation of new genotypes and cytotypes. Evidence for the following evolutionary phenomena is currently available: (1) allopolyploidization, (2) homoploid hybrid formation, and (3) establishment of viable offspring by means of fusion of highly aneuploid gametes, which resulted from irregular meiotic divisions. In contrast, there is no strict evidence demonstrating the occurrence of autopolyploidization. *Boechera* experts have just started to integrate evolutionary concepts derived from molecular analyses into taxonomic research, and profound changes, with respect to species definition and circumscription, are to be expected in the near future. The identified modes of reproduction and evolutionary phenomena will contribute to the development of a theoretical and practical basis on which future taxonomic classifications and species concepts can be based.

Key words: agamospermy, *Boechera*, Brassicaceae, chromosome number, embryology, evolution, hybridization, karyology, pollen, population genetics, speciation, taxonomy.

The genus *Boechera* Á. Löve & D. Löve (Böcher's rock cress, Brassicaceae) has recently been recognized as a monophyletic isolated lineage (Koch et al., 1999; Koch, 2003) of North American–Greenlandic distribution, based on DNA sequence variation. The genus, which according to current taxonomic treatments (Rollins, 1993; Mulligan, 1996) comprises about 80 species, was formerly included within a broadly defined genus *Arabis* L. s.l. The latter is highly polyphyletic, containing species that are to be treated within the genera *Streptanthus* Nutt. (*A. petiolaris* A. Gray; I. Al-Shehbaz, pers. comm.), *Pennellia* Nieuwl. (e.g., *A. microsperma* Rollins; Al-Shehbaz, 2003), *Arabidopsis* Heynh. (O'Kane & Al-Shehbaz, 1997; Koch et al., 1999), *Fourraea* Greut. et Burdet (*A. pauciflora* (Grimm) Garcke; Koch et al. 1991, 2001), *Pseudoturritis* Al-Shehbaz (*A. turrita* L.; Koch et al. 1999, 2001), and *Turritis* L. (*A. glabra* L.; Koch et al., 2001). Based on the occurrence of two base chromosome numbers within *Arabis* s.l., Löve and Löve (1975) proposed the splitting of this broadly distributed genus into a New World taxon ($x = 7$, *Boechera*) and a second group chiefly of Old World distribution ($x = 8$, *Arabis* s.s.). This classification is supported by several morphological characters (Al-Shehbaz, 2003).

Recently Koch et al. (2005) identified a basal phylogenetic split within the Brassicaceae based on

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the occurrence of a pseudogenic tandem repeat found in the $trn\mathrm{L}_{(UAA)}$-$trn\mathrm{F}_{(GAA)}$ intergenic spacer. The repeated region constitutes a synapomorphy supporting a clade comprising the cardaminoid and arabidopsoid Brassicaceae (Heenan et al., 2002), with the genus *Boechera* placed at an evolutionarily derived position. The base chromosome numbers of sister taxa (*Crucihimalaya* Al-Shehbaz, O'Kane & R. A. Price and *Capsella* Medik.) and of branches emerging deeper from its evolutionary history are primarily $x = 8$. The phylogenetic pattern of cytological differentiation shown in Figure 1 provides strong evidence that the ancient base chromosome number of the taxon, which has given rise to the cardaminoid and arabidopsoid Brassicaceae was indeed $x = 8$. This ancestral base chromosome number is furthermore supported, at least in the arabidopsoid lineage, by the high degree of collinearity between the genomes of *Capsella rubella* Reut. and *Arabidopsis lyrata* subsp. *petraea* (L.) O'Kane & Al-Shehbaz (both $2n = 16$) as has been demonstrated by Koch & Kiefer (2005) (cf. fig. 1). Consequently, the $x = 7$ condition in *Boechera* must be considered to be a derived condition resulting from chromosome reduction. Comparative map-based studies are now available that explain structural changes in plant genomes, as has been done in the case of the highly derived chromosome set ($x = 5$) of *Arabidopsis thaliana* (L.) Heynh. (Acarkan et al., 2000). Such genetic maps have not yet been constructed for *Boechera*, although several hundred microsatellite loci have been isolated and the construction of linkage maps is currently underway (T. Mitchell-Olds, pers. comm.). From recent advances in the mapping of Brassicaceae genomes (Kowalski et al., 1994; Lagercrantz, 1998; Kuittinen et al., 2004; Lysak et al., 2003, 2005), we can expect these data to provide resolution on the structural changes responsible in the origin of the distinct $x = 7$ base chromosome set characterizing *Boechera*.

The genus *Boechera* has long been known for its extensive morphological polymorphism, which is thought to have resulted from the combined evolutionary forces of hybridization, polyploidy, and apomixis (Rollins, 1966, 1983; Dobeš et al., 2004a, 2004b, 2006). However, during the past seven decades research activities have focused mainly on three taxa: *B. divaricarpa* (A. Nelson) Á. Löve & D. Löve, *B. holboellii* (Hornem.) Á. Löve & D. Löve, and the only one that is a proven monophyletic species, *B. stricta* (Graham) Al-Shehbaz (formerly *Arabis drummondii* A. Gray). Only fragmentary data on the evolutionary patterns and processes from the remaining species of this genus have been gathered.

The aim of this article is to synthesize the available data on karyology, palynology, population genetics, reproductive mode, and associated cytological pathways on a genus-wide level, with a special focus on the resulting chromosomal composition of gametes and its evolutionary significance in the establishment of new genotypes and cytotypes, respectively.

Because of the enormous taxonomic difficulties within this genus, one has to take into account that many past documentations refer to incorrectly identified material. For this reason, we are introducing a web-based gateway for ongoing *Boechera* and Brassicaceae research (<http://ephedra.hip.uni-heidelberg.de/brassicaceae>). Through this gateway, all re-examinations of vouchers referring to cytological references provided during the past seven decades will be accessible. This is also true for the summarized and discussed data of this review. In the future, we aim to open and link several other database resources on crucifer research (Ihsan Al-Shehbaz and co-workers, Michael Windham and Donovan Bailey).

Although the cytological and embryological mechanisms and phenomena addressed here may be invalid in a taxonomic context, they are applicable to evolutionary questions (i.e., they mirror evolutionary processes occurring in the genus *Boechera*). Consequently, they contribute to a theoretical and practical basis on which we can build evolutionary models and on which we can develop taxonomic and species concepts in the near future. Therefore, we recommend reading of this review in an evolutionary context, even though we, for practical reasons, have to refer to the only currently available, but highly artificial, taxonomic classification.

SOMATIC CHROMOSOME NUMBERS

Karyological studies in *Boechera* have focused mainly on the estimation of chromosome numbers, and karyotypic analyses of their small chromosomes (3–6 μm) have recently been performed (Sharbel et al., 2004, 2005). These studies have revealed several structural peculiarities of the *Boechera* genome. For instance, Mulligan (1964: 1517) stated that diploid "native North American *Arabis*" (genus *Boechera*) species have one pair of somatic chromosomes with satellites, while triploid plants of *A. divaricarpa* A. Nelson had three somatic chromosomes with satellites. Sharbel et al. (2004) described the diploid genome of *B. holboellii* to be composed of autosomes, which have median to subterminal centromere positions.

Another conspicuous feature is the presence of aneuploid chromosomes (or chromosome fragments) that were mainly observed in *Boechera holboellii* (Table 1a and 1b) and that can be differentiated from the normal autosomes by their conspicuously smaller size (Böcher, 1947; Mulligan, 1964; Packer, 1964;

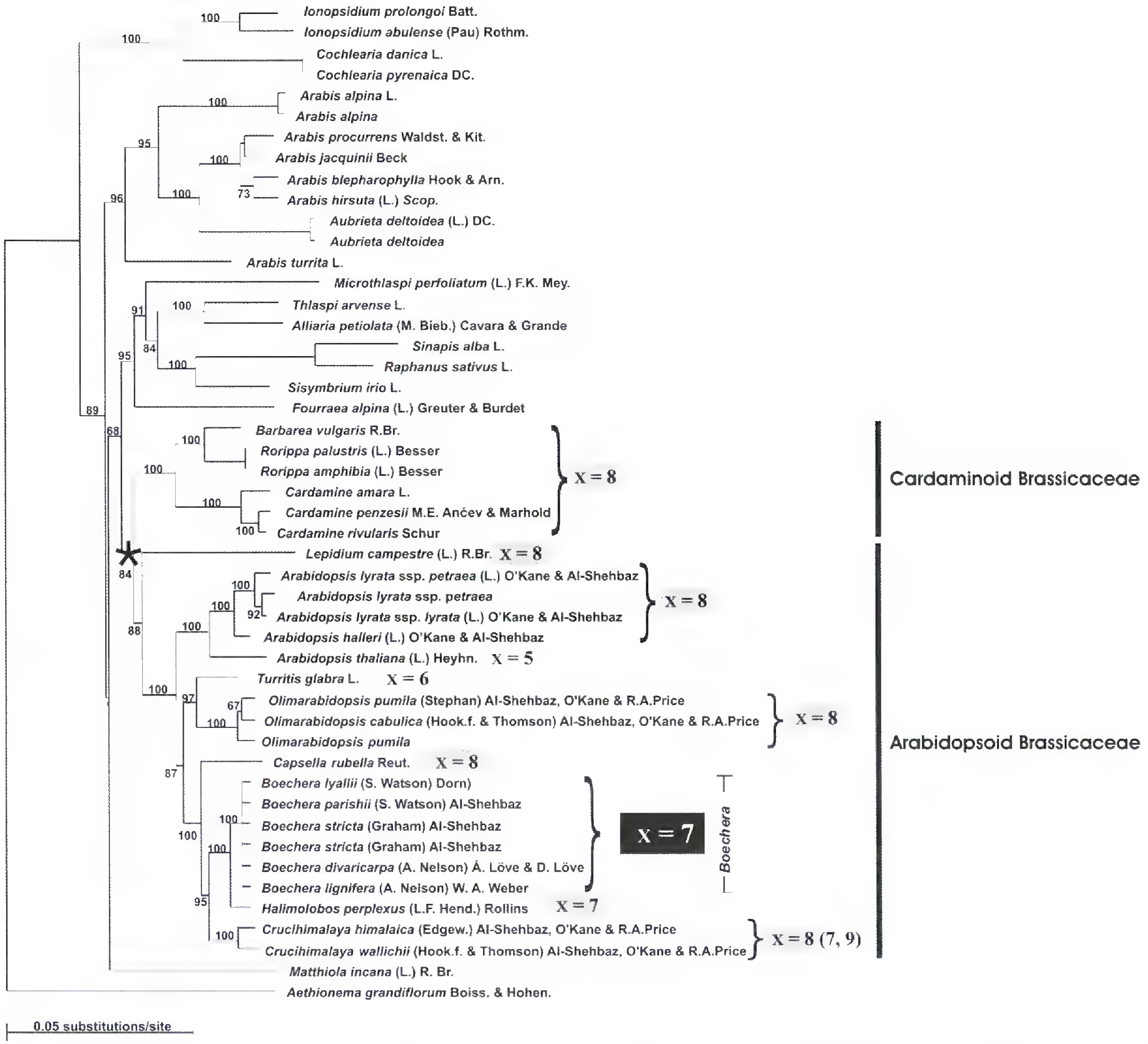
Ionopsidium prolongoi Batt.
Ionopsidium abulense (Pau) Rothm.
Cochlearia danica L.
Cochlearia pyrenaica DC.
Arabis alpina L.
Arabis alpina
Arabis procurrens Waldst. & Kit.
Arabis jacquinii Beck
Arabis blepharophylla Hook & Arn.
Arabis hirsuta (L.) Scop.
Aubrieta deltoidea (L.) DC.
Aubrieta deltoidea
Arabis turrita L.
Microthlaspi perfoliatum (L.) F.K. Mey.
Thlaspi arvense L.
Alliaria petiolata (M. Bieb.) Cavara & Grande
Sinapis alba L.
Raphanus sativus L.
Sisymbrium irio L.
Fourraea alpina (L.) Greuter & Burdet
Barbarea vulgaris R.Br.
Rorippa palustris (L.) Besser
Rorippa amphibia (L.) Besser
Cardamine amara L.
Cardamine penzesii M.E. Ančev & Marhold
Cardamine rivularis Schur
x = 8
Cardaminoid Brassicaceae
Lepidium campestre (L.) R.Br. x = 8
Arabidopsis lyrata ssp. *petraea* (L.) O'Kane & Al-Shehbaz
Arabidopsis lyrata ssp. *petraea*
Arabidopsis lyrata ssp. *lyrata* (L.) O'Kane & Al-Shehbaz
Arabidopsis halleri (L.) O'Kane & Al-Shehbaz
x = 8
Arabidopsis thaliana (L.) Heyhn. x = 5
Turritis glabra L. x = 6
Olimarabidopsis pumila (Stephan) Al-Shehbaz, O'Kane & R.A.Price
Olimarabidopsis cabulica (Hook.f. & Thomson) Al-Shehbaz, O'Kane & R.A.Price
Olimarabidopsis pumila
x = 8
Arabidopsoid Brassicaceae
Capsella rubella Reut. x = 8
Boechera lyallii (S. Watson) Dorn)
Boechera parishii (S. Watson) Al-Shehbaz
Boechera stricta (Graham) Al-Shehbaz
Boechera stricta (Graham) Al-Shehbaz
Boechera divaricarpa (A. Nelson) Á. Löve & D. Löve
Boechera lignifera (A. Nelson) W. A. Weber
x = 7
Boechera
Halimolobos perplexus (L.F. Hend.) Rollins x = 7
Crucihimalaya himalaica (Edgew.) Al-Shehbaz, O'Kane & R.A.Price
Crucihimalaya wallichii (Hook.f. & Thomson) Al-Shehbaz, O'Kane & R.A.Price
x = 8 (7, 9)
Matthiola incana (L.) R. Br.
Aethionema grandiflorum Boiss. & Hohen.
100
100
100
95
100
100
96
73
91
100
84
95
100
89
68
100
100
100
100
84
100
100
92
88
100
100
97
67
100
87
100
100
100
95
100
0.05 substitutions/site

Figure 1. Phylogenetic relationships among cruciferous plants based on *chs* and *mat*K sequence data (redrawn from Koch et al., 2001, reprinted with permission from The Botanical Society of America). The asterisk marks the initiation of a *trn*F pseudogene tandem repeat at the basis of the cardaminoid and arabidopsoid Brassicaceae (Heenan et al., 2002; Koch et al., 2005). The phylogenetic distribution of chromosome numbers among the cardaminoid and arabidopsoid Brassicaceae is taken as evidence that the base chromosome number of the progenitor of these lineages was most likely $x = 8$.

Table 1a and 1b (pp. 520–524). Evidence for sexual and apomictic reproduction taken from karyological investigations, analyses of pollen, estimation of seed fertility, and population studies reported for *Boechera* species and affined taxa. Species names are according to Rollins (1993); taxonomic ranks are according to Mulligan (1996). Although most species are treated within genus *Boechera* (cf. Al-Shehbaz, 2003, for synonomy with *Arabis*), four *Arabis* species are also included. *Arabis petiolaris* and *A. serotina* probably have to be transferred to other genera (see below) but were included for clarity. The remaining two *Arabis* species have not yet been formally transferred to *Boechera*, but are included based on the similarity in genus cytology. Superscript index numbers refer to the publications listed in the footnote. Gray fields signify features associated with apomictic reproduction and species showing evidence of apomixis.

Table 1a

Taxon	Chromosome number[a]					Meiosis[c]		Type of Pollen[f]		Fertility		Population genetic studies[i]
	2n = 14	2n = 14 ± 2	2n = 21	2n = 21 ± 2[b]	others	Sex[d]	Apomeiosis[e]	Tetrads	Monads Dyads	% Pollen viability[g]	% Seed Set[h]	
***Arabis boivinii*[Y] G.A. Mulligan**			2[4]			☒	? M[4]					
Arabis depauperata[Y] A. Nelson & P.B. Kenn.	1[4]					M[4]						
Arabis petiolaris[X]					2n = 28[8]							
Arabis serotina[W]	1[20]											
B. breweri (S. Watson) Al-Shehbaz	1[7]									**90 ± 9[21]**	71 ± 29[21]	
B. canadensis (L.) Al-Shehbaz	1[10]											
B. cobrensis (M.E. Jones) Dorn	1[3]					P[3]						
B. constancei (Rollins) Al-Shehbaz	1[7]											
B. crandallii (B.L. Robinson) W.A. Weber	3[3.6]					P[3]						Iso - Sex[22]
***B. demissa* (Greene) W.A. Weber**			1[6]			☒						
B. divaricarpa	15	1, B: 1	9	4, B: 1	2	See Table 1b for details						
B. fendleri	7[3.6.19]					P[3], M[19]	P[3]					
			2[3.8]	1[8]		☒	P[3]					
B. glaucovalvula (M.E. Jones) Al-Shehbaz	1[9]					P[9]						
B. gunnisoniana	1[3]					P[3]	E, P[40]					
			1[22]			☒			D[22]	58[22]	38[22]	Iso - Apo[22]
B. holboellii	85	4, B: 5	28	4, B: 6	7	See Table 1b for details						
***B. inyoensis* (Rollins) Al-Shehbaz**			1[7]	1[7]		☒						
B. laevigata (Muhlenberg ex Willdenow) Al-Shehbaz	5[3.13.14.18.38]					P[3.14.18] M[13.38]						
***B. lemmonii* (S. Watson) W.A. Weber**	3[6.10]											
			1[4]			☒	? M[4]				abundant[4]	
B. lignifera	3[3.22]					P[3]		T[22]		**91[22]**	46[22]	Iso – Apo?[22]
B. lyallii			5[4,10,21]			☒	? M[4], ? P[21]			34 ± 28[21]	65 ± 22[21]	
***B. microphylla* (Nuttall) Dorn**	2[3.10]					P[3]						
	1[3]	1[29]					P[3,29]		D[29]			

Table 1a and 1b. Continued.

B. parishii	1[9]					P[9]						
B. pendulina (Greene) W.A. Weber	1[29]							uniform[29]				
B. perennans (S. Watson) W.A. Weber	2[3.7]					P[3.7]						
B. perstellata (E. Braun) Al-Shehbaz	2[6.15]					M[6.15]						
B. platysperma (A. Gray) Al-Shehbaz	1[21]					P[21]				**91 ± 11[21]**	77 ± 25[21]	
B. puberula **(Nuttall) Dorn**	1[4]		1[4]			M[4]	? M[4]					
B. pulchra **(M.E. Jones ex S. Watson) W.A. Weber**	3[7,9]		2[7]			P[9]						
B. repanda (S. Watson) Al-Shehbaz	1[3]					P[3]						
B. schistacea (Rollins) Dorn	1[29]							uniform[29]				
B. selbyi **(Rydberg) W.A. Weber**			1[8]									
B. shortii (A. dentata)	2*n* = 12[13]					M[13]						
B. sparsiflora **(including *Arabis columbiana*)**	9[2,4]	1[35]	3[4,7,21]	6[4,7,16,21,29] B: 1[7]	2*n* = 46 2*n* = 32[17]*	P[2],M[4]	? M[4] ?E[29], P[16,29] ? M[4]		M, D[29] Z	**88-97[2]** 62 ± 46[21]	73 ± 25[21]	
B. stricta	20		2	1	1	See Table 1b for details						
B. subpinnatifida (S. Watson) Al-Shehbaz	3[8.21.37]					P[8.37]				**95 ± 2[21]**	50 ± 16[21] 45 ± 18[21]	
B. williamsii (Rollins) Dorn												Mor–uniform[5]
B. crandallii x B. holboellii			1[6]									
B. divaricarpa x B. holboellii				2[28,29]			P[28,29]		D[29]			
B. holboellii x B. stricta			1[2]				? P[2]			19[2]		

Table 1a and 1b. Continued.

Table 1b

Taxon	Material denotation	Somatic Chromosome number	Meiosis^{c}		Type of Pollen^{f}		Fertility		Population genetic studies^{i}	Citation^{j}
			Sex^{d}	Apomeiosis^{e}	Tetrads	Monads Dyads	% Pollen viability^{g}	% Seed Set^{h}		
B. divaricarpa		[15] ("n = 15")		**P**						3
		16	P [?]							3
		14	M							4, 5; 11
		15; **21**; 28	×	? M						4
		14; **22**	×							6
		13 + 2B; 14; **20 + 2B**; **21**; 28	×							10, 31, 33
		21; **22**	×	? P			64 ± 38	65 ± 19		21
B. holboellii	Colorado	**21**	E (0%--5%)	**E (95%--100%),P**			95			1
including	Greenland	14	E (11%--55%), P	**E (45%--89%), P**	Mixture of reduced and unreduced pollen		33			1
Arabis holboelli Hornemann	Greenland	**21**	E (2%--17%)	**E (83%--98%)**			50			1
A. collinsii Fernald	12 biotypes	14	P				**92--99**			2
A. exilis A. Nelson	2 biotypes	28; 28 + 1f	P				**89--94**			2
A. pendulocarpa A. Nelson		14	P, M							3, 17, 36
A. pinetorum Tidestr.		[14] ("n = 14")								3
A. retrofracta Graham		[21] ("n = 21")	×	**P**						3
		14	M (mostly)	? M (few)						4
		c. 21	×	? M		**D**				4
		21	×	? M; **P**						4, 29
		15	M	? M						4
		c. 22	×	? M						4

Table 1a and 1b. Continued.

	14							Mor–uniform	5; 6, 10, 28, 29, 30, 31, 32
	21								6, 10, 26, 28, 29
	21 ± 1								7
	14 (*n* = c.8 in a few cells)	P	? P						8
	13 + 2B								10
	20 + 2B								10
	14 + 1B, 14 + 1f								10, 12, 30
	14 (*n* = 7; 9)	P	? P			32 ± 18	72 ± 13		21
	28				D	85	46		22
	21 + 1f				D	(42)88--95	38--83.3	Mol – Apo^{22,23}	22
	21				D	(3)73--97	(15)57--77		22
	14			T	D	86--92	32--51		22
HBH	21 + 1f		E, P	T	M, D				24, 25, 26
HBH	14	(E)	E		M, D				27
Disko 1	14								26, 27, 28
Disko 2	21		E, P						27, 28, 29
S. Str. 10	14	E, P	E, P	T	D, M				26, 27, 28
S. Str. 3	21		E, P	T	D, M				27, 28
S. Str. 9	21		E, P		D	Plant fertile, one completely sterile shoot			27, 28
Eqaluit	21		E, P		D				26, 27, 28
Alaska	14	E, P		T			Normal		26, 27, 28
No 3090	28								28
No 2820	35 (of accidental origin)								28
No 2820	2x		P		D				28

Table 1a and 1b. Continued.

	No 6	21 + 1f	☒	P		D				29
	No 6	17					shrivelled			29
	No 12	14	M		T					29
	No 16	21 + 1B or 20 + 2B	☒							29
	No 20, A-Batch	14; 14 + 1B; 16; 17			T	D				29
	No 20, B-Batch	21; 21 + 1B	☒	M	T	D				29
		22	☒							39
B. stricta		14	M, P				**98**			2, 4
(*A. drummondii*)		14	P							3, 8, 34
		21	☒	? M						4
		14	☒							5; 6, 10, 31
		20	☒							10
		28	P		Reduced					29

[a] Number of published chromosome reports classified according to ploidy levels and the occurrence of additional chromosomes. Somatic triploidy was taken as evidence for apomictic reproduction. [b] B indicates that the additional chromosome(s) was a B chromosome. [c] E and P denote female or male gametogenesis, respectively; "M" signifies that the gender of the gametophyte has not been stated by the authors.* The count $2n = 32$ for *B. sparsiflora* was based on a misidentified true *Arabis* (M. Windham, pers. comm.) and, therefore, has not been considered in further statistics provided in the manuscript. [d] Cell division listed in this column showed characteristics indicative for sexual reproduction: complete, regular pairing of chromosomes at meiosis (bivalents); regular meiotic division resulting in gametes with reduced chromosome number. Crisscrossed fields underline that, so far, evidence for normal regular meiosis was not demonstrated in association with triploidy. [e] Divisions listed in this column showed characteristics indicative for apomictic reproduction: asyndesis, apomeiosis, gametophyte with unreduced chromosome number. A question mark (?) indicates irregular, often incomplete, pairing of chromosomes and irregular divisions, resulting in gametophytes with irregular chromosome numbers. [f] D, M, and T denote dyadic, monadic, and tetradic pollen. "Reduced" and "Unreduced" refer to chromosome number. [g] The percentage of stainable pollen grains is provided; estimates of fertility of pollen produced by sexuals are given in bold. [h] Values provided by Vorobik (1985) refer to fruit set, while those given in Roy (1995) are the percentage of germinating seeds. [i] Evidence for sexual ("Sex") and apomictic ("Apo") reproduction taken from population studies; "Iso" refers to population genetic studies using isozymes; "Mor" are observations on the variability of morphotypes. [j] Records of identical information content have been merged. [w] This species may belong to the genus *Pennellia* (Al-Shehbaz, pers. com.). [x] This species may belong to the genus *Streptanthus* (Al-Shehbaz, pers. com.; Koch & Dobeš, unpubl. data). [y] This species has formally not been transferred to *Boechera*. Therefore, it is nomenclaturally considered as an *Arabis*. [Z] Pollen was highly variable among flowers: (1) some flowers put out few dyads and monads, while the majority of pollen grains were of irregular size and shape; (2) some flowers formed dyad pollen only. Numbers in superscript

Table 1a and 1b. Continued.

refer to the following publications: 1 = (Naumova et al., 2001); 2 = (Johnson, 1970); 3 = (Rollins, 1941); 4 = (Mulligan, 1996); 5 = (Rollins, 1983); 6 = (Rollins, 1966); 7 = (Rollins & Rüdenberg, 1971); 8 = (Rollins & Rüdenberg, 1977); 9 = (Rollins & Rüdenberg, 1979); 10 = (Mulligan, 1964); 11 = (Taylor & Brockman, 1966); 12 = (Packer, 1964); 13 = (Smith, 1938); 14 = (Easterly, 1963); 15 = (Rollins, 1960); 16 = (Raven et al., 1965); 17 = (Taylor & Taylor, 1977); 18 = (Kovanda, 1978); 19 = (Ward, 1983); 20 = (Wieboldt, 1987); 21 = (Vorobik, 1985); 22 = (Roy, 1995); 23 = (Roy & Rieseberg, 1989); 24 = (Böcher, 1947); 25 = (Böcher, 1938); 26 = (Böcher & Larsen, 1950); 27 = (Böcher, 1951); 28 = (Böcher, 1954); 29 = (Böcher, 1969); 30 = (Sharbel et al., 2004); 31 = (Löve, 1982); 32 = (Löve, 1979); 33 = (Löve, 1975); 34 = (Löve, 1976); 35 = (Löve, 1969); 36 = (Dalgaard, 1988); 37 = (Stace, 1998); 38 = (Stace, 1989); 39 = (Stace, 1999); 40 = (Tsakin et al., 2004).

Böcher, 1969; Johnson, 1970; Rollins & Rüdenberg, 1971). Recent work has demonstrated these extra chromosomes to be B chromosomes (Camacho et al., 2000), as is evidenced by their interspecific origin, variable morphology, high content of heterochromatin, and independent evolution from the autosomes (Sharbel et al., 2004). Interestingly, the B chromosomes have a wide geographic distribution and can be found in both diploid and triploid *Boechera* (Sharbel et al., 2005). Furthermore, DNA sequence analyses of B chromosome alleles suggest a single origin for these elements (Sharbel et al., 2004).

However, chromosome number is the most intensively studied karyological property of *Boechera* that has been studied (Böcher, 1938; Smith, 1938; Rollins, 1941; Böcher, 1947; Böcher & Larsen, 1950; Böcher, 1951, 1954; Rollins, 1960; Easterly, 1963; Mulligan, 1964; Packer, 1964; Raven et al., 1965; Rollins, 1966; Taylor & Brockman, 1966; Böcher, 1969; Löve, 1969; Johnson, 1970; Rollins & Rüdenberg, 1971; Löve, 1975, 1976; Rollins & Rüdenberg, 1977; Taylor & Taylor, 1977; Kovanda, 1978; Rollins & Rüdenberg, 1979; Löve, 1979, 1982; Rollins, 1983; Ward, 1983; Vorobik, 1985; Wieboldt, 1987; Dalgaard, 1988; Stace, 1989; Roy & Rieseberg, 1989; Roy, 1995; Mulligan, 1996; Stace, 1998, 1999; Naumova et al., 2001; Sharbel et al., 2004; Tsakin et al., 2004), the data for which is now available for 36 species (34, if *Arabis petiolaris* and *A. serotina* E. S. Steele are excluded from the genus), comprising about half of the *Boechera* species recognized in the monography of Rollins (1993). Much of the current taxonomy in *Boechera* may be expected to be subject to far-reaching revision in the near future (Windham et al., 2004), but there is no doubt that $x = 7$ constitutes the common and probably only base chromosome number of the genus. The only taxon deviating from this rule may be *B. shortii* (Fernald) Al-Shehbaz (*A. dentata* Torr. & Gray), for which a single count of $n = 6$ II (bivalents) was published by Smith (1938). A total of 289 chromosome records (excluding counts reported repeatedly and for taxa probably belonging to other genera) are currently available. This total is strictly limited to those taxa currently recognized as *Boechera* s.s. (cf. Al-Shehbaz, 2003) and to direct observation of chromosome number rather than inference by flow cytometry or pollen size. In order to minimize propagation of any error in species recognition, we do not provide here the original data, which were collected in a taxonomic context. Instead, in addition to Table 1a and 1b, an annotated and fully documented database summarizing published data on karyology, pollen, and population genetics is available at <http://ephedra.hip.uni-heidelberg.de/brassicaceae>. (In both data files we applied the taxonomy and nomenclature suggested in Rollins, 1993, Mulligan, 1996, and Al-Shehbaz, 2003, respectively.) These databases are continuously updated in regard to taxonomic identification. In addition, two databases that might be useful for additional information will be accessible in the future. A chromosome number database is compiling most references of chromosome counts in cruciferous plants (Warwick & Al-Shehbaz, 2006), and a crucifer taxonomy database is listing many new taxonomic treatments (Warwick et al., 2006). The most recent findings on *Boechera* are not considered in these databases, but updates will follow continuously.

From an evolutionary point of view, several obvious features with respect to chromosome numbers can be deduced from this overview. (1) Diploidy is very common, comprising 190 out of 289 chromosome records. (2) Polyploidy is primarily expressed on the triploid ploidy level ($2n = 3x$), although higher ploidy levels are also found in a limited number of reports ($N = 12$). (3) Aneuploid chromosomes occur frequently and are associated with both even and odd-ploids. (4) Provided that a sufficient number of counts were carried out, many of the individual taxa exhibited a variety of cytotypes, including diploids, polyploids, and aneuploids. However, it remains to be verified whether the latter feature represents a significant cytological pattern of differentiation or whether it merely reflects an erroneous assignment of accessions and artificial taxonomic classification of populations.

The unevenness in sampling must be considered, as 67% of the data (195 out of 289 records) describe the karyological differentiation in accessions attributable to three species only (*Boechera divaricarpa*, *B. holboellii*, and *B. stricta*), while 69% of the species so far investigated (25 out of 36) are represented by one to three chromosome counts only.

In addition, some of the karyological data require revision or reinterpretation, taking into consideration any ambiguities that may have resulted from varying degrees of synapsis and nonreduction in chromosome numbers during meiosis (Böcher, 1951; Johnson, 1970). As will be discussed in more detail in the next section ("Development of gametophytes"), the karyotypic irregularities common to *Boechera* embryogenesis and pollen development are associated with agamospermy and hybridization and may have resulted in several unreduced gametophytic chromosome numbers being published as haploid ones (Rollins, 1941), including *B. divaricarpa*, a count of $n = 15$; *B. fendleri* (S. Watson) W. A. Weber, 14 and 21; and *B. holboellii*, 14 and 21 (cf. comments in Böcher, 1969; Mulligan, 1996). Such high ploidy levels probably do not exist in *Boechera*. A true duplication of the somatic chromosome number within an individual has nonetheless been reported from the somatic tissues of *B. sparsiflora* (Nutt.) Dorn, $2n =$ ca. 23 and ca. $2n = 46$ (Böcher, 1969), the apparent outcome of an endomitotic event.

Aneuploidy has been reported (in terms of species numbers) eight times. However, it can be inferred from the observed pattern of cytological differentiation within *Boechera holboellii* that autosomal aneuploidy may be the exception rather than the rule. Thus only 9 somatic chromosome numbers out of the 139 records ascribed to this species were aneuploidies not attributable to B chromosomes. The infrequency of aneuploids may reflect the need for proper chromosome balance for pseudogamous endosperm formation in apomicts. In contrast, the flow cytometric data of Sharbel and Mitchell-Olds (2001) demonstrate high frequencies for aneuploid chromosomes of two size classes (cf. fig. 5 in Sharbel & Mitchell-Olds, 2001), the larger of which likely represents the autosomal aneuploid chromosome. This larger aneuploid chromosome was restricted to a single (or very few) chloroplast haplotype (Sharbel & Mitchell-Olds, 2001) and therefore may reach very high frequencies in specific geographic regions or genetic backgrounds. The smaller of the aneuploid elements is a B chromosome, which has a wide geographic distribution (Sharbel et al., 2005) and can be found in divergent genetic backgrounds (Sharbel et al., 2004), but has what appears to be a single origin based on B allele sequencing (Sharbel et al., 2004). This nonrandom distribution of Bs in apomictic individuals only, in conjunction with its wide distribution (i.e., evolutionary maintenance), implies that it either has a meiotic drive mechanism (cf. Camacho et al., 2000) or that it is somehow associated with apomictic reproduction.

While the *Boechera holboellii* genome frequently contains additional chromosomes (both B and aneuploid chromosomes), natural populations are typically composed of both diploid and triploid cytotypes (Roy, 1995; Sharbel et al., 2005). Recent cytological, palynological, and molecular research has provided us with the opportunity to describe and understand this pattern in more detail. Using pollen morphology and microsatellite allelic variation, Dobeš et al. (2004a) described diploidy and triploidy among 77 accessions of this species, whereas Sharbel and Mitchell-Olds (2001) and Sharbel et al. (2005) demonstrated the occurrence of B chromosomes in both of these cytotypes using flow-cytometry and karyology. These results are concordant with those collected from light microscopical investigation of chromosomes. For example, Naumova et al. (2001) carried out chromosome counts on 37 plants from

Colorado and Greenland representing eight separate seed batches and one bulked seed collection but found exclusively euploid diploids and euploid triploids. Because no tetraploid individuals have been identified in these mixed populations, it is hypothesized that environmental effects (e.g., cold shock) may trigger the production of unreduced gametes, which eventually contribute to triploid zygote formation (Sharbel & Mitchell-Olds, 2001; Sharbel et al., 2005). The frequent and repeated generation of triploid cytotypes within natural populations and in different genetic backgrounds (Sharbel & Mitchell-Olds, 2001; Sharbel et al., 2005) provides further evidence that unreduced gamete production is common in this genus (cf. Böcher, 1951). Triploids were demonstrated to be of allopolyploid origin (Sharbel et al., 2004; Dobeš et al., 2004a), and in this case it is unclear how genome dosage and subsequent phenotypic effects differ between independently derived triploid lineages (Sharbel et al., 2005). Autopolyploidy was suggested based on the frequent sharing of haplotypes between diploids and triploids (Sharbel & Mitchell-Olds, 2001). It was further shown that certain diploid and triploid cytotypes share identical to genetically highly similar nuclear ribosomal ITS sequence types (Dobeš et al., 2004a). However, based on microsatellite allelic variation, polyploid individuals always were found to show some extent of heterozygosity (Dobeš et al., 2004a). Therefore, we have so far no strict evidence for autopolyploidy in *Boechera*, neither on the genotypic nor on the species level. It may be speculated, then, that polyploidization can only be triggered in diploid hybrids via the formation of unreduced gametes (see discussion below). It is furthermore unknown whether reported ploidy levels higher than $3x$ are the result of similar phenomena (i.e., zygote formation from unreduced gametes in mixed populations of *B. holboellii*) or if these cytotypes are of a different evolutionary history. Although based on much smaller datasets, cytological characteristics similar to those in *B. holboellii* are also exhibited by several other *Boechera* species (cf. Table 1a and 1b; <http://ephedra.hip.uni-heidelberg.de/brassicaceae>).

A second type of cytological differentiation with respect to chromosome numbers was encountered in several species so far observed to be represented by euploid diploids only. We mention here explicitly two examples. First, *Boechera stricta*, which is also a good example to discuss possible limits of morphology-based species recognition in *Boechera*. The majority of chromosome counts identified this species as a diploid one. However, triploids were also found among the investigated accessions (Sharbel & Mitchell-Olds, 2001; Sharbel et al., 2005). Subsequent morphological investigations (Windham & Al-Shehbaz, unpublished data) have shown that the triploids identified as *B. stricta* have actually undergone hybridization with another species. Support for these hypotheses was recently found using microsatellite allelic markers (cf. Dobeš et al., 2004a). Another conspicuous example is represented by a certain morphotype of *B. holboellii*, *B. holboellii* var. *retrofracta* (= *B. retrofracta* (Graham) Á. Löve & D. Löve). A significant proportion of diploid specimens assigned to this variety showed covariation of characteristic plastid and nuclear sequence polymorphisms (Dobeš et al., 2004a). These genotypes were interpreted to constitute a basal evolutionary unit within a highly polymorphic and taxonomically artificial *B. holboellii*. These examples demonstrate that karyologically heterogenous species may actually be an amalgamation of different taxa, at least some of which can be characterized by single chromosome numbers and a restricted number of genotypes, respectively.

Finally, based on our recent evaluation of data, we may define a third type of karyological differentiation represented by taxa that so far proved to comprise triploids only. Solely triploid taxa seem to be extremely rare according to current taxonomic classification of *Boechera*. Nevertheless, future taxonomic concepts can integrate knowledge on values of apomictic reproduction of triploids or the age of apomictic clones, which may ultimately lead to the recognition of cytologically more uniform species.

DEVELOPMENT OF GAMETOPHYTES

Our cytological knowledge on the development of embryosacs and male gametogenesis in *Boechera* comes mainly from the work of Tyge W. Böcher (Böcher, 1951, 1954, 1969) and contributions by Naumova et al. (2001) and Tsakin et al. (2004). Apomictic *Boechera* are diplosporous (the embryo develops from an unreduced embryosac mother cell) similar to the *Taraxacum* L.-type, and it appears that pseudogamy is maintained given that fertilized endosperm has been identified in both diploids and triploids (Böcher, 1947, 1951; Naumova et al., 2001; Tsakin et al., 2004). Based mostly on pollen analyses, sexuality has been shown to be associated with complete chromosome bivalent formation at meiosis, followed by regular heterotypic and homotypic divisions, to produce gametes with reduced chromosome numbers while pollen was tetradic and usually of high viability (Böcher, 1951). In contrast, apomictic gametogenesis involved either complete asyndesis or irregular pairing of chromosomes during meiosis I, followed by the formation of a restitution nucleus to result in gametes with unreduced chromosome numbers (Böcher, 1951). Pollen was either of the dyadic or monadic type. Apomeiotic development was often accompanied by pathways exhibiting approximately normal meiosis with varying numbers of univalents, resulting in tetrad pollen with varying chromosome numbers and relatively low fertility (Böcher, 1951; Sharbel et al., 2005). Although these latter developmental traits can be considered to be associated with apomixis, the occurrence of meiotic abnormalities probably cannot be equated with apomictic reproduction itself.

Complete analyses of embryological developmental stages have been performed from a limited number of accessions of *Boechera holboellii* or infraspecific members of this complex (Rollins, 1993) originating from Greenland ("Disko"; "Botanical Garden of Copenhagen"; "Eqaluit"; the Sondre Strømfjord) and two localities in the western United States (Colorado; Washington state) (Böcher, 1951, 1969; Naumova et al., 2001). In addition to these studies, Tsakin et al. (2004) analyzed the embryology of one accession from Colorado identified as *B. gunnisoniana* (Rollins) W. A. Weber. Any other indications of apomixis were obtained from karyological investigations of isolated stages of female gametogenesis, population genetic studies (Rollins, 1983; Roy, 1995), observations on reproductive biology (Johnson, 1970; Vorobik, 1985; Roy, 1995), and analysis of pollen formation (e.g., Böcher, 1951; Dobeš et al., 2004a). Therefore, embryological studies have so far been performed on two species, *B. holboellii* s.l. and aff. *B. gunnisoniana*, from a few geographic areas only.

From these considerations we can estimate the importance and frequency of the particular reproductive modes in *Boechera*. We have summarized published data on karyology, pollen and seed formation, fertility, and within-population differentiation in Table 1a and 1b and in the supplementary database (available on request and at <http://ephedra.hip.uni-heidelberg.de/brassicaceae>). Important traits that enable the differentiation of apomixis and sexuality, including pollen formation, degree of chromosome pairing at meiosis, and chromosomal reduction, also were considered, although nuclei showing irregular chromosome pairing at meiosis may not necessarily be indicative of apomeiosis. Given this uncertainty, these irregular pairing constellations were not considered sufficient to judge the reproductive mode of a given individual. Furthermore, we used the argument that stable triploidy should be associated with apomixis (cf. Sharbel & Mitchell-Olds, 2001), because no known sexual pathway in *Boechera* could explain the maintenance of uneven ploidy levels. As *Boechera* are annuals, biennials, and short-lived perennials without any effective modes of vegetative propagation, agamospermy seems to be the only possible mechanism through which triploidy can be inherited. However, triploidy does not necessarily mean obligate apomixis, and these cytotypes can potentially give rise to offspring of different ploidy levels.

Although apomixis is a female trait (i.e., asexual reproduction through egg cells), it can be inferred from observations of pollen formation because apomeiosis is reflected in both the male and female components of *Boechera* (Böcher, 1951). For example, Böcher (1951: 33) demonstrated correspondence between the modes of male and female gametogenesis in several accessions ("Disko 2," "HBH 3x," "S.Str. 3," sterile shoot in "S.Str. 9," and "S.Str. 10"). This correlation was even observed in specific inflorescences in facultative apomictic *Boechera* (i.e., those producing seed both through sex and apomixis). For instance, a high degree of chromosome pairing during female meiosis was identified in flowers from the same inflorescence in which highly synaptic meiosis was identified in pollen mother cells ("HBH 3x" and "S.Str. 10" material) (Böcher, 1951). Furthermore, high degrees of syndesis were correlated with seed sterility and decreased pollen viability on a particular branch of the inflorescence in the triploid "S.Str. 9" accession (Böcher, 1951).

Taking the above-mentioned traits into consideration, we were able to find evidence of apomictic reproduction in accessions assigned to 17 species. Apomixis was associated with (1) diploidy, triploidy, or rarely tetraploidy; (2) aneuploidy and euploidy; (3) formation of dyadic and monadic pollen or tetradic pollen of usually low viability; and (4) low levels of genetic or morphologic differentiation within population. Apomixis is rarely the only mode of reproduction in any *Boechera* species, and hence nine of the 17 species for which apomixis was identified are likely amphi-apomictic complexes (cf. Table 1a and 1b), which presumably have to be split into separate taxonomic entities. However, amphi-apomictic lineages have to be expected given the report of decreased values of apospory in *Boechera* (cf. Naumova et al., 2001 and discussion below).

Despite the accumulated amount of chiefly descriptive data regarding reproduction in *Boechera*, we still have poor knowledge of the genetics and inheritance of apomixis in this genus. A first approach to infer the genetic basis of apomixis was recently made by Sharbel et al. (2004), who hypothesized that the B chromosomes may somehow be associated with the expression of the apomictic trait. Considering that the B chromosome in geographically and genetically distinct apomictic lineages is composed of a contiguous genomic region, Sharbel et al. (2004) proposed that it is evolutionarily important because it has not been lost by genetic drift, as would be expected for a neutral or deleterious B chromosome (Camacho et al., 2000). However, cases of apomeiotic formation of gametophytes in the absence of extra chromosomes have been reported (Rollins, 1941; Böcher, 1951, 1954), and thus Sharbel et al. (2004) argued that such an apomixis factor could also be expected to be present in autosomes. It should be noted that several experts in *Boechera* taxonomy have some doubt on some counts provided by Rollins (1941).

The gathered data (e.g., Johnson, 1970; Rollins, 1941; Mulligan, 1996; Rollins & Rüdenberg, 1977, 1979) provided evidence for the existence of sexual species that were mostly diploid, exhibited complete chromosome pairing and meiotic chromosomal reduction, and were characterized by high pollen viability and seed fertility. These taxa (e.g., *Boechera stricta*) are characterized by euploid and diploid chromosome numbers and produce *Polygonum* L. –type embryosacs via the meiotic pathway (Naumova et al., 2001; Tsakin et al., 2004). Recent taxonomic studies estimate the actual number of sexual diploid *Boechera* to be at least 70 (Al-Shehbaz & Windham, pers. comm.). Evidence for what is possibly purely sexual reproduction in polyploids has been provided for three accessions only (*B. holboellii*, $2n = 28$; $28 + 1f$, Johnson, 1970; *B. stricta*, $2n = 28$, Böcher, 1969). In contrast, and as expected from theoretical considerations, the reduction of chromosome numbers associated with regular bivalent formation was rarely observed in triploids.

Interestingly, syndesis in *Boechera* is not only genotypically determined, i.e. reflecting homology of chromosomes, but it also can be modificative. Böcher (1951) explained the facultative occurrence of syndesis to be controlled by an unknown physiologic factor. This is best shown by the fact that apomeiosis and meiotic pairing can occur side by side and in definite parts within a single inflorescence (Böcher, 1951; Naumova et al., 2001). Additionally, changes in temperature or environmentally induced differences in rate of growth of plants were shown to make *B. holboellii* change from apomeiotic to meiotic development, or at least change from fertility to sterility. Therefore, the available data indicate that we can expect various reproductive pathways to co-occur frequently among plants of a population and even within single individuals.

POLLEN FORMATION

Male gametogenesis has been favored over the study of the development of female gametophytes by several authors (e.g., Rollins, 1941; Easterly, 1963; Raven et al., 1965; Johnson, 1970; Vorobik, 1985), because pollen is easily and abundantly available. Pollen viability can routinely be inferred from simple staining procedures (Vorobik, 1985; Naumova et al., 2001), pollen grain shape and size (Böcher, 1951; Roy, 1995; Dobeš et al., 2004a; Sharbel et al., 2005), and germination experiments (Böcher, 1951). Furthermore, correlations between the size of pollen grains and their DNA content have repeatedly been shown in several other genera (Ehrendorfer, 1949; Stepánková, 1993; Trávnícek & Vinter, 1999; Vilhar et al., 2002; Lihová & Marhold, 2003), and thus relative pollen ploidy can also be estimated.

Apomictic *Boechera* is a highly interesting and promising system to study pollen cytology, as gametes with highly variable chromosome numbers can be produced by single individuals (Böcher, 1951, 1969). For example, tetradic, dyadic, and monadic pollen have been observed within specific parts of a single inflorescence, with monad pollen mainly observed in the lowermost portion of the inflorescence, while dyads prevailed in its middle part (Böcher, 1951, 1969). Both types of pollen resulted from apomeiotic gamete development, which was often followed by the formation of a restitution or contraction nucleus. In any of these apomeiotic pathways, one (heterotypic division; formation of dyads) or both (heterotypic and homotypic division; monads) of the divisions corresponding to those in normal meiosis were abandoned (cf. Böcher, 1951: 21 seq.). As a consequence, gametes can be produced with either unreduced chromosome numbers or with double the somatic chromosome number of their bearers, as has been shown in somatic triploids that produce both triploid and hexaploid pollen (e.g., Böcher, 1951; Kravtchenko et al., 2001). Double pollen grains possessing four generative and two vegetative nuclei have also been observed (Böcher, 1951).

In contrast, the formation of tetradic pollen generally involved some degree of chromosome pairing followed by a reductional division. Gametes produced via this pathway by individuals exhibiting evidence for apomixis were reported to contain highly variable chromosome numbers, the result of cytological irregularities during their formation (cf. Böcher, 1951, 1969). Gametes containing chromosome numbers that deviated from those expected under a scenario of either regular meiotic division or complete (apomeiotic) nonreduction have been observed by several authors (Rollins, 1941; Böcher, 1951; Johnson, 1970; Vorobik, 1985).

A conspicuous case was described by Böcher (1969), who identified two different classes of synaptic (i.e., syndetic) triploids based on pollen size data and chromosome configurations during meiosis I, including those that produced only large pollen and those that produced a bimodal distribution of smaller pollen size. Those triploids producing a single class of relatively large pollen often showed regular metaphase I with 10 bivalents plus one univalent, rather than the seven trivalents or corresponding lower numbers of bivalents and univalents (Böcher, 1951). Such pairing formations have subsequently been observed by several authors (Böcher, 1951, 1969; Johnson, 1970) and have recently been supported by pollen analyses (Sharbel et al., 2005). Regular bivalent formation in triploids was hypothesized to be the outcome of structural chromosome mutations (Böcher, 1951), although it is difficult to understand how such mutations (a random process) could convergently lead to a similar pattern of synapsis in different triploid lineages (Sharbel & Mitchell-Olds, in prep.). Although these cytological irregularities led to a considerable decrease in pollen viability (Böcher, 1951; Vorobik, 1985), a limited number of gametes were still fertile and capable of participating in the production of viable offspring (Böcher, 1969; see later discussion).

Nogler (1984) suggested that alleles responsible for apomixis may act as, or are linked to, recessive lethal factors, as would be expected in an asexual organism (Kondrashov, 1985). The accumulation of recessive lethal mutations in apomicts over time would therefore lead to decreasing fertility of haploid gametes. Indeed, Naumova et al. (2001) observed a mixture of reduced and unreduced pollen grains in apomictic diploid *Boechera holboellii*, while only unreduced female and male gametes contributed to mature seed. These results suggested, as stated by these authors, that there was selection against haploid gametes. Alternatively, proper dosage of an apomixis factor may be important, and haploid gametes may simply be unable to express this trait. However, tetradic pollen produced by sexuals was usually of high quality (Johnson, 1970; Vorobik, 1985; Roy, 1995) and obviously was formed by normal and undisturbed meiosis.

Data on the chromosomal composition of male gametes have recently been supplemented with morphological analyses of pollen. Although the size of pollen grains appeared highly variable among species (Vorobik, 1985; Roy, 1995) and within individuals (Böcher, 1951), Dobeš et al. (2004a) demonstrated that pollen size can be used to identify diploid sexuals in *Boechera holboellii* and *B. stricta*. Furthermore, plants capable of apomictic reproduction were characterized by a multimodal distribution of pollen sizes (Böcher, 1951). These various classes of pollen size corresponded to tetradic, dyadic, and monadic pollen, respectively, as well as to two distinct sizes of pollen mother cells. Böcher (1951) concluded that formation of pollen with unreduced chromosome number is frequent in apomictic *B. holboellii*, taking place in some diploids and all triploids. This observation is also in agreement with the results of Roy (1995), Naumova et al. (2001), and Kravtchenko et al. (2001), who also found formation of dyads as well as of monads to be common.

ESTABLISHMENT OF OFFSPRING

Two main reproductive pathways allowing the stable and constant inheritance of chromosome number in specific cytotypes have been identified in *Boechera*: regular meiosis in euploid diploids and tetraploids and agamospermy in a variety of cytotypes. Sexual *Boechera* appear to be highly self-compatible and hence inbreeding is very common, as has been inferred through isozyme patterns (Roy, 1995) and microsatellites (Dobeš et al., 2004a). The floral biological investigations of Johnson (1970) on nine biotypes assigned to *B. holboellii* and *B. lyallii* (S. Watson) Dorn, *B. sparsiflora*, and *B. stricta* demonstrated that each was self-pollinating and was adapted for some outcrossing by insects (cf. Rollins, 1971). Johnson (1970) also identified different biotypes co-occurring at single sites and discovered evidence for mechanical and ethological (i.e., preference of different insects to certain biotypes) isolation mechanisms between them. Although the above observations provide clear evidence for strong isolation between lineages, repeated introgression between sexual taxa and also between sexuals and apomictics is likely frequent at both the intra- and interspecific levels (Koch et al., 2003; Sharbel et al., 2004; Dobeš et al., 2004a, 2006). Thus, hybrids between *Boechera* species have been repeatedly obtained in crossing experiments (Johnson, 1970; Vorobik, 1985; Roy, 1995) and observed in the field (e.g., Rollins, 1983, 1993; Mulligan, 1996). At present, the importance of hybrid speciation in the radiation and diversification of the genus is widely accepted (Windham et al., 2004). While hybridization in *Boechera* was previously thought and demonstrated to be associated with polyploidization and the apomictic stabilization of new genotypes (Böcher, 1951; Sharbel & Mitchell-Olds, 2001; Koch et al., 2003; Dobeš et al., 2004a), the report of diploid and sexual *B. divaricarpa*, a hybrid between *B. holboellii* (and probably other species) and *B. stricta*, may provide evidence that homoploid sexual hybrids have played a similarly important role as polyploidization in the establishment of new species.

As with regular meiosis, apomixis potentially allows for the stable inheritance of chromosome number in the course of reproduction. Furthermore, agamospermy resembles autogamy, a special case of sexual reproduction often encountered in *Boechera* that leads to offspring having the same genotype as their parent (Roy, 1995). However, a fundamental difference between agamospermy and apomixis is that apomixis maintains heterozygosity and stabilizes polyploid, aneuploid, and hybrid genomes. The population genetic consequences of apomictic reproduction include the coexistence of different and potentially crossable but clearly distinct morphotypes within populations (Rollins, 1983), in addition to low intrapopulation level differentiation (Roy, 1995; Naumova et al., 2001). Whereas agamospermy in *Boechera* is almost never obligate, facultative apomixis has frequently been demonstrated (Böcher, 1951, 1969; Naumova et al., 2001), with average values for apomeiosis during female gametophyte development ranging from 73% to 98% in *B. holboellii* (Naumova et al., 2001). Naumova et al. (2001) also observed apomictic embryos with somatic ploidy levels double that of their mothers, the result of fusion between unreduced male and female gametes and therefore deviating from normal parthenogenetic development. The above-mentioned mechanisms, in addition to variable chromosomal synapsis (Böcher, 1951, 1969), imply that apomixis plays a significant and specific role in the establishment of new genotypes. Additional genetic variation can be expected from crossing-over events between chromosomes (i.e., automixis) at various pairings, which were reported to take place even in apomictic plants (Böcher, 1951, 1969).

The role of apomeiotic development of gametes in the origin of triploids, which represent the most common ploidy level in *Boechera*, is particularly well understood. Böcher (1951) previously pointed out that amphi-apomictic diploids could easily produce triploid offspring by fusion of reduced and unreduced gametes. The frequent and repeated establishment of triploid genotypes within and among various evolutionary lineages has been confirmed by Sharbel and Mitchell-Olds (2001) and Sharbel et al. (2005) through the use of chloroplast sequence variation. Dobeš et al. (2004a) and Sharbel et al. (2004) have furthermore established that these polyploid cytotypes are the outcome of crossing events between similar to largely diverged genotypes. By analogy, agamospermous diploids might pass the apomictic trait without a change in ploidy levels to genetically recombined offspring when occasional fusion of two reduced gametes takes place, and this may occur between two apomicts or even between an apomict and a sexual individual. However, the establishment of triploids may have also involved diploid pollen from tetraploids. Although somatic tetraploids were found to be extremely rare in *Boechera*, they may be evolutionarily significant. Thus Verduijn et al. (2004) demonstrated that tetraploid dandelions (genus *Taraxacum* L., Asteraceae) give rise to mainly triploid offspring in crosses with diploid sexuals. A similar scenario may apply to *Boechera*, as was recently hypothesized by Dobeš et al. (2004a). Unlike *Taraxacum*, in which tetraploids are the only cytotype putting out diploid pollen with high frequency, unreduced gamete production appears to be relatively common among diploid *Boechera*.

While the origin of triploids can be explained by the above processes, the establishment of diploid apomicts is much less obvious. However, an intriguing possibility is that triploids that produce bimodal pollen sizes (i.e., pollen with 14 + B or a complement of 7 chromosomes) may be the source of apomictic diploid 2C + B *Boechera holboellii* (Böcher, 1969; Sharbel et al., 2005). This explanation would allow shifts between diploids and triploids and facilitate regular genetic exchange between these cytotypes (cf. Verduijn et al., 2005).

Evidence for exemplary hybrid origin in *Boechera* is provided and discussed in Dobeš et al. (2006). The origin of diploid and triploid cytotypes, both of which are the most frequently encountered in *Boechera*, is concordant with the chromosomal composition of gametes. However, the scarcity of tetraploids and higher ploidy levels in natural populations suggests that natural selection acts against their formation, with genomic balance or stabilization through apomixis. We can say so because it appears that selection does not have a significant impact on gametes, considering that dyad and monad pollen were shown to have morphological integrity (Dobeš et al., 2004a) and high viability (Böcher, 1951; Roy, 1995). Furthermore, unreduced pollen was shown to successfully fertilize the central cell of embryosacs, which is required for pseudogamous endosperm development (Naumova et al., 2001; Tsakin et al., 2004).

CONCLUSIONS

We can expect that the processes described above would lead to a large variety of new genotypes and cytotypes with distinct chromosome numbers. Furthermore, these new types may potentially become fixed through asexual reproduction, as meiotic irregularities were chiefly observed in individuals carrying the apomictic trait. However, the reproductive success of such genetically recombinant and genomically heterogeneous progenies has never been demonstrated in the long term, and their evolutionary significance is not fully understood yet. It will be a promising field of future experimental research to correlate structural changes of the genome in hybrid offspring with fitness measures over several generations, which we presumably can do as soon as linkage maps become available (cf. Rieseberg et al., 2000). Estimation of the performance of hybrid genotypes and cytotypes will enable us to assess whether hybrids are merely byproducts of evolution or whether they themselves actively drive diversification in this genus.

The North American *Boechera* group is a highly complex species aggregate within Brassicaceae. However, the group itself is clearly defined and restricted to North America, which is also true for most of its close relatives. For this reason, it is realistic that the ongoing projects focusing on its taxonomy, systematics, evolutionary history, and phylogeography will provide a good framework to understand speciation and evolution in this notoriously difficult group. Additional research on molecular mechanisms of apomixis, genome structure, and chromosomal synteny and collinearity will provide data that bridge evidences from cytology and embryology to species diversity.

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